Synthesis of a Phthaloylglycine-Derived Strigol Analogue and Its Germination Stimulatory Activity toward Seeds of the Parasitic Weeds Striga hermonthica and Orobanche crenata

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The newly designed strigol analogue Nijmegen 1 (rac 7) was prepared in high overall yield starting from N-phthaloylglycine. This relatively simple analogue exhibits high bioactivity in the stimulation of germination of seeds of the parasitic weeds Striga hermonthica and Orobanche crenata. Nijmegen 1 was resolved in its enantiomers 7 and ent 7 by using the homochiral latent D-rings ent 11 and ent 12. The enantiomers 7 and ent 7 show significant differences in germination activity.

Keywords: Striga; Orobanche; germination; strigol analogue

INTRODUCTION

The devastating parasitic weeds Striga and Orobanche cause severe reductions in food crop yield of several graminaceous and leguminous crops in tropical and semitropical areas of the eastern hemisphere (Muselman, 1987; Parker and Riches, 1993). A strict requirement for the germination of the seeds of these parasitic weeds is exposure to a chemical substance that is usually present in the root exudate of a potential host plant (Press et al., 1990; Butler, 1995). An attractive control strategy for the eradication of infested fields is the concept of suicidal germination, i.e. introduction of a germination stimulating agent into the soil prior to sowing to induce germination of the parasitic seeds in the absence of a host plant (Eplee, 1975). The first known naturally occurring germination stimulant, (+)-strigol (1), was isolated from the root exudate of the false host cotton (Gossypium hirsutum L.) (Cook et al., 1966, 1972). Recently, (+)-strigol (1) was also identified in the root exudates of the Striga host plants maize (Zea mays L.) and proso millet (Panicum miliaceum L.) (Siame et al., 1993). In addition, some structurally closely related “strigolactones” (Butler, 1995) have been identified in the root exudates of other Striga hosts, viz. sorgolactone 2 (Hauck et al., 1992) and alectrol 3 (Müller et al., 1992).

However, strigolactones 1-3 (Figure 1) are not suitable for weed control purposes, because their structures are too complicated to allow synthesis in an economically feasible manner. Therefore, several studies aimed at synthetic analogues with a relatively simple structure but with high germination stimulatory activity (Johnson et al., 1976, 1981; Vail et al., 1990; Bergmann et al., 1993; Mangnus et al., 1992a; Zwanenburg et al., 1994). These studies mainly focused on the ABC-part of the strigolactones. In this part of the molecule a considerable structural variation is allowed to retain high biological activity. On the basis of these observations, a tentative molecular mechanism (Scheme 1), which accounts for the onset of the biochemical cascade leading to germination, has been proposed (Mangnus and Zwanenburg, 1992a). According to this mechanism the bioactive phoreresides in the vinyl ether part of the D-ring.

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Figure 1. Strigolactones 1-3 and some active analogues.

Important examples of highly potent synthetic analogues include 4 (GR7) and 5 (GR24) (Johnson et al., 1981; Mangnus and Zwanenburg, 1992b; Mangnus et al., 1992b). The latter, having an aromatic A-ring, is especially highly relevant, as its stimulatory activity is comparable to that of strigol (Bergmann et al., 1993) and its preparation is much easier than that of strigol (Mangnus et al., 1992b). An even less complicated analogue is compound 6, derived from γ-phenyl-γ-butyrolactone. This analogue, which lacks the B-ring, is almost as active as GR24 (Mangnus et al., 1992a). A
Scheme 1. Proposed Molecular Mechanism Involved in Germination

The essence of the molecular mechanism is not affected. Its GR analogues (m, 2H, 2arom H), 3.77 (s, 3H, OC), 3.16 (m, 1H, J = 2.8 Hz), 2.85 (m, 1H, J = 3.6 Hz), 2.38 (m, 1H, J = 3.6 Hz), 1.77 (s, 3H, OC), 1.65 (m, 2H, J = 2.8 Hz). The residue was triturated with diisopropylether. Almost pure rac 9 (660 mg, 64%) was isolated as a white solid by filtration and washing with diisopropyl ether. An analytical sample was obtained by recrystallization from 2-propanol. mp 151–152 °C; [α] D -22° (c 0.2, CHCl3); H NMR (CDCl3, 400 MHz) δ 1.97 (br s, 3H, C); MS [EI, m/z, rel intensity (%)] 343 (M1+), 2.74, 246 (C15H17NO3)+, 100, 97 (C8H5O2)75.0. Anal. Calcd for C22H19NO7: C, 64.54; H, 4.68; N, 3.43. Recrystallization from dichloromethane (three times), drying (MgSO4) and concentration in vacuo. Recrystallization from toluene gave pure 9 (59.3 g, 80%) as a pale yellow powder, with physical properties identical with those reported previously (Sheehan and Johnson, 1954).

Methyl 2-(1,3-Dioxo-1,3-dihydroisoxandol-2-yl)-3-(4-methyl-5-oxo-2,5-dihydrofuran-2(R)-yloxy)acrylate (rac 7). Potassium tert-butoxide (372 mg, 3.32 mmol) was added to a cooled (0 °C) and stirred solution of Sheehan aldehyde 9 (745 mg, 3.02 mmol) in DMF (10 mL) at room temperature under nitrogen. Then chlorofuranone (480 mg, 3.62 mmol) in DMF (3 mL) was gradually added. The mixture was stirred at room temperature over a weekend. DMF was removed in vacuo, and the residue was dissolved in a mixture of water and ethyl acetate. The organic layer was separated, and the aqueous phase was extracted with ethyl acetate (two times). The combined organic layers were washed with water (two times), dried (MgSO4), and concentrated in vacuo. The oily residue was triturated with diisopropyl ether. Almost pure rac 7 (660 mg, 64%) was isolated as a white solid by filtration and washing with diisopropyl ether. An analytical sample was obtained by recrystallization from 2-propanol. mp 151–152 °C; [α] D -22° (c 0.2, CHCl3); H NMR (CDCl3, 400 MHz) δ 1.97 (br s, 3H, C); MS [EI, m/z, rel intensity (%)] 343 (M1+), 2.74, 246 (C15H17NO3)+, 100, 97 (C8H5O2)75.0. Anal. Calcd for C22H19NO7: C, 64.54; H, 4.68; N, 3.43. Recrystallization from dichloromethane (three times), drying (MgSO4) and concentration in vacuo. Recrystallization from toluene gave pure 9 (59.3 g, 80%) as a pale yellow powder, with physical properties identical with those reported previously (Sheehan and Johnson, 1954).

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Methyl 2-(1,3-Dioxo-1,3-dihydroisoxandol-2-yl)-3-(6(R)-methyl-5-oxo-4-oxatricyclo[5.2.1.02,6]dec-8-en-3(R)-yloxy)acrylate (rac 12). This compound was prepared in the same way as 12, starting from Sheehan aldehyde 9 (60 mg) and rac enantiomer to 9 (53 mg, 40%) as a colorless oil, which failed to crystallize [α] D +24° (c 0.15, CH2Cl2). H NMR and mass data were the same as for compound 12.
Synthesis and Bioactivity of a Glycine-Derived Strigol Analogue

Methyl 2-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-[4-methyl-5-oxo-2,5-dihydrofuran-2(5)-yloxy]acrylate (ent 7) was prepared in the same way as described for 7, starting from ent 12 (230 mg, 0.56 mmol). Yield: 43 mg, 31% of ent 7 as a colorless oil, which failed to crystallize. $[\alpha]_D = 128^\circ$ (c 0.15, CH$_2$Cl$_2$). $^1$H-NMR and mass data were the same as for compound 7.

Biological Activity. Seeds. Seeds of Striga hermonthica (Del.) Benth [from Sorghum bicolor (L.) Moench] and Orobanche crenata Forsk. (from Vicia faba L.) were harvested in Sudan in 1988 and in Egypt in 1991, respectively, and were stored in the dark at room temperature until use in germination tests.

Preparation of Test Solutions. A compound to be tested was weighed out very accurately to the amount of 10 mg, dissolved in 10 mL of acetone p.a., and diluted with demineralized water to 100 mL. Aliquots of this stock solution were further diluted with water to obtain test solutions containing 2, 1, 0.1, and 0.01 mg/L test compound and 0.2, 0.1, 0.01, and 0.001% (w/v) acetone, respectively.

Bioassays. For surface sterilization seeds of S. hermonthica and O. crenata were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then thoroughly rinsed with water and dried overnight.

For conditioning the sterilized seeds were spread on glass fiber filter paper dishes (8-mm diameter; approximately 30–70 seeds per disk) in Petri dishes, wetted with water, and stored in the dark for 14 days at 20 °C for Orobanche seeds and at 30 °C for Striga seeds. Then the conditioning water was removed and replaced by 100 µL of test solution per disk. After incubation for 24 h (Striga) and 5 days (Orobanche) in the dark at indicated temperatures, the germination percentage was determined under a microscope. Seeds were considered to be germinated if the radical protruded through the seed coat.

In each test series aqueous solutions with 0.1, 0.01, and 0.001% (w/v) acetone were used as negative control. Test solutions of the stimulant GR24 (as a 1:1 diastereomeric mixture at concentrations of 1, 0.1, and 0.01 mg/L) were used as positive controls. All tests were performed in duplicate, and in each test the germination percentages were determined on 12 disks per treatment.

For full details of the bioassay, see Mangnus et al. (1992c).

RESULTS AND DISCUSSION

The key step in the synthesis of rac 7 involves coupling of aldehyde 9 with 5-chloro-3-methyl-2(5H)-furanone (10). This aldehyde 9 was prepared by condensation of methyl N-phthaloylglycinate (8) with methyln formate using metallic sodium (Scheme 2).

This procedure, which closely resembles that described by Schutz (1978), is superior to that originally reported by Sheehan and Johnson (1954). It should be noted that 9 is a stable, crystalline compound, which can be stored for several years. The coupling reaction with butenolide 10 (Scheme 2) proceeded in high yield, and purification was readily accomplished by recrystallization. It is important to note that only one geometrical isomer was obtained. The correct geometrical isomer could not be deduced unambiguously by spectroscopic means, and therefore an X-ray diffraction analysis was undertaken (Beurskens et al., 1994). The structure of 7 is depicted in Figure 2, showing that the Z-isomer was obtained.

Next, the preparation of the individual enantiomers of 7 was attempted, using enantiomerically pure tricyclic chloroacetones 11 and ent 11 as the D-ring precursors (Scheme 3). The stereoselective synthesis of 11 and ent 11 and their use in the preparation of the single isomers of strigol analogues has been reported recently (Thuring et al., 1995).

The coupling reactions of Sheehan aldehyde 9 with 11 and ent 11 did not proceed as smoothly as was observed for the corresponding GR7 analogues (Thuring et al., 1995). As a result of the relatively poor nucleophilicity of the enolate anion derived from 9, a higher reaction temperature was required, which caused concomitant decomposition of 11 and ent 11. The cycloreversion of 12 and ent 12 was performed in o-dichlorobenzene at 180 °C to give 7 and ent 7, respectively, in moderate yields. The ee values of both enantiomers were >98%, as was determined by $^1$H-NMR analysis using the chiral shift reagent Eu(hfc)$_3$.

Biological Evaluation. The germination stimulatory activity of Nijmegen 1 (rac 7) was assayed using seeds of S. hermonthica and O. crenata spp. In each bioassay, GR24 was included as a positive control. The procedure enables a comparison between results obtained in different test series. This is important, since the response of seeds of parasitic weeds, especially S. hermonthica, varies considerably from test to test. In addition, the activities of enantiomers 7 and ent 7 were determined using seeds of O. crenata spp. The results were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then thoroughly rinsed with water and dried overnight.

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Figure 2. PLUTON-generated drawing of X-ray crystal structure of Nijmegen 1 (rac 7).

Table 1. Germination Percentages for Seeds of S. hermonthica after Exposure to Aqueous Solutions of Strigol Analogues GR24 and Nijmegen 1 at Concentrations of 1 and 0.01 mg/L.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Stimulant</th>
<th>% Germination ± SE</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>GR24 (5)</td>
<td>45.5 ± 13.5</td>
</tr>
<tr>
<td>2</td>
<td>Nijmegen 1 (rac 7)</td>
<td>40.3 ± 1.7</td>
</tr>
<tr>
<td>3</td>
<td>45.5 ± 13.5</td>
<td>40.3 ± 1.7</td>
</tr>
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*Activities are indicated as germination percentages after treatment of the seeds with test solutions at 1 mg/L and 0.01 mg/L. Germination percentages given are the mean ± SE of two replicate tests. $^a$ Value is not significantly different from germination percentages obtained in the control (without stimulant).
are collected in Tables 1 and 2. It was beyond the aim of this study to establish complete dose-response curves, implying that the data obtained allow only an interpretation in a qualitative sense.

The data in Table 1 (S. hermonthica spp.) reveal that rac Nijmegen 1 exhibits considerable activity at the higher concentration of 1 ppm, whereas it is practically inactive at a concentration of 0.01 ppm. Similarly, in the stimulation of O. crenata spp. seeds, rac 7 has shown a bioactivity comparable to that of GR24 at higher concentrations (entry 1, Table 2). Comparison of the germination percentages exerted by enantiopure 7 and ent 7 (entries 2 and 3, Table 2) reveals that the former is considerably more active. Thus, the absolute stereochemistry at C-2' in the D-ring should be the R-configuration to germinate a maximum number of seeds. This configuration is the same as in natural (+)-strigol. This result is in agreement with previous conclusions from comparative studies of the bioactivity of all stereoisomers of GR7 (Mangnus and Zwanenburg, 1992b) and of some stereoisomers of strigol (Bergmann et al., 1993), namely, that the most active stereoisomer has the R-configuration at C-2' in the D-ring.

From the results presented above, it may be concluded that phthaloylglycine-derived strigol analogue rac 7 is a potent germination stimulant of seeds of S. hermonthica and O. crenata spp. Moreover, optically active 7 with the “natural” configuration in the D-ring has a stimulatory activity comparable to that of GR24 for O. crenata. The charm of this particular stimulant is the fact that its racemic preparation is very simple and that it can be carried out without any chromatographic separation, which makes it an attractive compound for large-scale preparations and accordingly for use in the suicidal approach in the weed pest control. Moreover, the achiral "ABC"-part in rac 7 enables a rapid evaluation of the structural variation in the D-ring on the stimulatory activity. Research in this direction is in progress.

It should be noted that our newly developed asymmetric route allows for the first time the synthesis of a strigol analogue, which is only chiral at the D-ring. The ease of preparation and the high bioactivity of this new germination stimulant warrant further studies to evaluate its activity and stability under soil conditions. Activities in this direction are in progress.

From a mechanistic point of view we can conclude that a possible interaction of the ABC-fragment with a receptor site is sterically and electronically not highly demanding.

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LITERATURE CITED


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